STUDIES ON BIOLUMINESCENCE.

XIII. Luminescence in the Cœlenterates.

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Introduction.

During a recent trip to the Pacific coast, the opportunity presented itself of studying light production in a number of cœlenterates and of making observations on other luminous forms. These studies were carried out at the Puget Sound Marine Station, Friday Harbor, Washington, and the author expresses his keen appreciation of the facilities afforded him there by Professor T. C. Frye, director of the laboratory.

We are indebted to Panceri (1) for most of our knowledge of luminescence among the cœlenterates. The present paper supplements his work and gives the facts in the case of certain forms found in Puget Sound.

Experiments on Hydromedusæ.

Seven species of hydromedusan jelly-fish were obtained at Friday Harbor, namely, *Æquorea forskalea*, *Mitrocoma cellularia*, *Phialidium gregarium*, *Stomatoca atra*, *Sarsia rosaria*, *Melicerta* sp. ? and an unidentified form. One scyphomedusan, *Cyanœa* sp. ?, is also occasionally to be collected. Of these only the first four produce light and only the first three are markedly luminous. The first five were very common forms, especially *Æquorea*, *Mitrocoma* and *Phialidium*, obtained in the morning from the laboratory float where they live in water of rather low temperature. Their appearance is somewhat capricious, however, and on several days only one or two were seen.

Æquorea and *Mitrocoma*, 1½ to 3 inches in diameter, and *Phialidium*, about 1 in. in diameter, produced light of a bright bluish-green color (but not so blue as the ostracod, *Cypridina*) from spots along the edge of the umbrella at the base of the

tentacles. No other regions of *Æquorea* or *Mitrocoma* phosphoresce, but at times faint light was to be observed coming from masses (gonads) along the four radial canals of *Phialidium*. *Melicerta* is weakly luminous about the manubrium and then only on rather vigorous rubbing between the fingers.

Examined with the microscope in the daytime, the margin of the umbrella of *Æquorea* discloses oval masses of yellow tissue corresponding in position with the luminous areas at night. In *Mitrocoma*, the yellow masses are much closer together forming an almost continuous line in some places. Since the luminous spots observed at night are also very close together in this form, it seems as if the yellow tissue must be luminous tissue. In *Cypridina* and the worm, *Tomopteris*, there are also very clearly visible yellow cells in the luminous gland but this is not true of the luminous organ of all forms. These yellow regions of the medusæ do not stain with neutral red, intravitam, a fact true also for the yellow cells of *Cypridina*. In *Phialidium* or *Stomatoca* yellow cells cannot be made out but this is possibly because of their small size.

Examined at night under the microscope, the luminous spots present a beautiful appearance. Under conditions which cause a cytolysis of the cell, such as addition of fresh water or saponin, one can clearly see that the light comes from granules which are rather large and have a definite boundary,—light discs. They are not mere points of light. They vary in size and will luminesce for some time, then flash out very brightly and the light intensity slowly fade. Sometimes there is the sudden appearance of a light disk and then gradual fading of the luminescence. In the luminous extract of *Cavernularia* (2) I have described a similar phenomenon, where, upon addition of fresh water, the light intensity suddenly increases, due to the flashing out of photogenic granules. Under the microscope the appearance is that of the starry sky.

By addition of saponin to the luminous tissue, we obtain a very bright light and this is the best method of exciting luminescence for examination with the spectroscope. This discloses a band of light extending from about $\lambda = .46\,\mu$ to $\lambda = .60\,\mu$. As far as I

was able to make out the limits are the same for both *Æquorea* and *Mitrocoma*, perhaps a somewhat narrower band for the latter.

The light of these jelly fish only appears on stimulation or on dissolution of the cell. It appears on handling or electrical stimulation, or when the jelly fish is carried by the current against some objects in the water. On merely touching a jelly fish one cannot observe that any luminous secretion is definitely thrown into the water as in the case of *Cypridina*, but on very gentle stroking of the edge of the umbrella a mass of luminous material comes off which adheres to the fingers, or on tossing an animal on the surface of the water, abundant luminous material is liberated which causes the sea water to luminesce. It appears that the luminous material comes off in the slime so commonly secreted by these organisms. A similar behavior is exhibited by the Pennatulid, *Cavernularia*. It would seem that this is to be interpreted as an extracellular luminescence, although not so marked a one, certainly, as that of *Cypridina*. There remains, however, the possibility that we have here cells very easily ruptured, with discharge of their contents into sea water.

The luminous material of *Æquorea*, *Mitrocoma* or *Phialidium* can be dried over $CaCl_2$ and will give a bright light when again moistened.

A strip of the margin of the umbrella of *Æquorea* or *Mitrocoma* is easily cut off with scissors, giving a mass of tissue containing as little non-luminous material as it is possible to obtain. If this is squeezed through four layers of cheesecloth, there is obtained a luminescent extract which glows for some hours. In one case the light was still visible after nine hours. This extract behaves just as a similar one prepared from *Cavernularia* (2). When the luminescence disappears on standing, the addition of fresh water, gentle heating or cytolytic agents such as saponin, sodium glycocholate, chloroform, ether, or NaCl crystals again calls forth the luminescence. Tannic acid, strychnin, or phloridzin do not cause the light to reappear. Once the light has been caused to disappear by addition of saponin or Na glycocholate powder, the further addition of fresh water will cause no more light to appear.

Isotonic cane sugar solution does not call forth the production

of light. The phenomenon is unquestionably one of cytolysis, by diminution of osmotic pressure, by heat, or by addition of specific substances. In the extract there are probably intact photogenic cells which dissolve with production of light. In addition I believe the solution of photogenic granules is also accompanied with the emission of light, because one can very easily see, under the microscope at night, the sudden appearance of a disc of light, too small to be the illumination of a cell, but capable of interpretation as the light from a single granule within the cell.

Extracts of *Æquorea* which should contain luciferase give no light with extracts of *Æquorea* which should contain luciferin. The same is true for *Mitrocoma* and for crosses of luciferin and luciferase of *Cypridina* with these two medusæ. Every attempt to demonstrate these substances has given negative results. The reasons for this are discussed in another paper, to appear shortly.

It is reported that many luminous forms produce no light in the daytime, the power only appearing with the approach of dusk or if the animals are kept in the dark for some time. This is true of some forms but not of these medusæ. The four kinds of luminous medusæ, *Æquorea*, *Mitrocoma*, *Phialidium* and *Stomatoca*, were collected in bright sunlight and brought by an assistant to the dark room where I had been adapting my eyes to the dark for one half hour. All four forms luminesced immediately on stimulation and just as brightly as at night. The ctenophore, *Bolina*, did not luminesce even after ten minutes, when brought into the dark from strong sunlight, but did luminesce after thirty minutes. There is no doubt that *Bolina*, a further discussion of which follows, is affected by sunlight but these four medusæ are certainly not. *Noctiluca* appears to have its luminescence inhibited by strong sunlight also.

Experiments on Ctenophores.

At Friday Harbor, three species of ctenophores occur, *Bolina* sp. ?, *Pleurobrachia* sp. ?, and *Beroë* sp. ?, but only the first was common during my stay. *Bolina* luminesces readily at night. *Pleurobrachia* did not luminesce even on crushing and *Beroë* only gives a diffuse flash of light on vigorous agitation.

The light of *Bolina* comes from cells along the swimming plates. According to Dahlgren (3), the luminous cells form a layer over the testis and ovary, along the water vascular canals. In the living animal I was unable to make out any yellow cells in this region, comparable to the yellow masses of *Æquorea* or *Mitrocoma*.

The light is of the same bluish-green color as the medusæ, but too faint and evanescent for a study of its spectrum.

Bolina is an exceedingly fragile ctenophore and contains much water and relatively little luminous material. The animals also appear to be easily fatigued and lose somewhat their power to luminesce on frequent agitation. Portions of the swimming plate tissue placed on a glass slide, as much of the water drained away as possible, and dried over $CaCl_2$ in the dark do not give light on again moistening with water. This is probably to be explained by the small amount of photogenic material present.

If Bolinas are pressed through four layers of cheesecloth there is obtained a luminescent solution which rather readily loses its power of luminescence. It again gives light on vigorous agitation or addition of cytolytic substances. It behaves as the extracts of medusæ and pennatulids. If fresh water is added, we have the appearance of dots of light just as in these extracts. The existence of luciferin and luciferase also cannot be demonstrated and extracts of *Bolina* give no light with *Cypridina* luciferin nor do heated extracts of *Bolina* give light with *Cypridina* luciferase.

Since the observations of Allman (4) it has been known that ctenophores would not produce light in the daytime. Peters (5) made quite a study of this in *Mnemiopsis* and found that mechanical stimulation accelerates the appearance of luminescence in darkness after previous exposure to light. The inhibition of luminescence is roughly inversely proportional to the intensity of the light which has previously illuminated them.

Bolina shows marked inhibition of luminescence as a result of previous illumination. Animals brought into a dark room from direct sunlight about 10 A.M. gave no light whatever on stimulation immediately or after five minutes, gave some light on stimulation after ten minutes, and a good luminescence after one half hour in the dark. The question at once arises as to the cause of this behavior. Are the cells incapable of being

stimulated after exposure to sunlight or do they fail to manufacture photogenic substances as a result of exposure to sunlight? One alternative supposes the cell to contain photogenic material which for some reason cannot be oxidized; the other, that no photogenic material is formed in the sunlight and the disappearance of that which has been formed. Some evidence can be obtained for the latter view by breaking up the cells of ctenophores which have been previously exposed to daylight. If no luminescence is produced the effect of light must be to prevent the manufacture of photogenic material. If luminescence occurs on breaking up of the photogenic cells previously exposed to daylight, the inhibitive actions of light must be on the stimulation mechanism.

If Bolinas, which have been previously exposed to daylight, are crushed through four layers of cheescloth, no light whatever appears during the crushing or on adding fresh water to cytolyse the photogenic cells. Similar Bolinas, kept in the dark for one half hour, give a bright luminescence under the same treatment. If this extract of crushed Bolinas, which had been previously exposed to sunlight, is allowed to stand in the dark for one half hour and then fresh water added, no light will appear. Whole Bolinas after sun illumination will again luminesce if kept in the dark for one half hour. This shows that there is no preformed photogenic material in sunlight exposed Bolinas and that none can be formed in crushed material even in the dark. The sunlight must therefore act to prevent the formation of photogenic substance rather than to prevent its oxidation on stimulation. Why sunlight causes the disappearance of photogenic material already formed is a question awaiting solution.

Experiments on a Sea Pen, *Ptylosarcus*.

Ptylosarcus is dredged at Friday Harbor in fairly deep water. Some of the specimens may be two feet long. The colony consists of a stalk without fronds buried in the sand and a stem with fronds that bear polyps only along the outer edge. The polyps, but not the surface of the fronds, are luminous. The stalk is not luminous but the stem has two luminous areas running the length of it and one non-luminous area between these.

The colony is non-luminous except when stimulated. Then a yellow greenish light appears of a more yellow hue than *Æquorea*. If the polyps are gently rubbed, a luminous slime comes off and the secretion can be seen in the sea water. The polyps, ground in sea water with sand, give a luminescent secretion which becomes very brightly luminescent on addition of fresh water, saponin and other cytolytic agents. The extract behaves exactly as a similar one prepared from *Cavernularia* and already described (2). In every way the behavior of *Ptylosarcus* agrees with that of *Cavernularia*.

It was impossible to demonstrate the presence of luciferin or luciferase in *Ptylosarcus*. The following "crosses" were also made, using extracts, which, from mode of preparation, should have contained *Ptylosarcus* luciferin and luciferase.

Ptylosarcus luciferase × *Ptylosarcus* luciferin—negative.
Ptylosarcus luciferase × *Æquorea* luciferin—negative.[1]
Æquorea luciferase × *Ptylosarcus* luciferin—negative.
Cypridina luciferase × *Ptylosarcus* luciferin—negative.
Ptylosarcus luciferase × *Cypridina* luciferin—negative.

Ptylosarcus brought into a dark room from direct sunlight was observed to luminesce on immediate stimulation and as brightly as at night. There is no inhibitive influence of light in this form.

Experiments on a Sponge, *Grantia*.

The question of luminosity in sponges is in rather of an unsettled state. Some observers have reported luminescence but Dahlgren (3) was inclined to attribute the light of a sponge obtained at Naples to luminous worms and protozoa living in its canals.

At Friday Harbor there exists a sponge, *Grantia* sp.?, one to three inches long, common on logs, piles, etc., in the sea water. If rubbed, a yellowish luminescence may be observed which can be obtained from all parts of the organism. If the sponge is crushed the luminescence is quite bright. Every individual of this kind of sponge examined showed luminescence, whereas another sponge, *Esperella* sp.?, living on *Pecten* shells, was not luminous. A few isolated dots of light only appeared on rubbing.

[1] Sometimes a faint light was observed whose significance is unknown.

Sponges kept in sunlight for one half hour gave as good a luminescence as those in the dark.

Whether this is a true luminescence or whether due to luminous organisms living on the sponge cannot be definitely stated. The sponge could not be stimulated to luminesce electrically (interrupted induced currents) under conditions when jelly-fish showed a good luminescence. Examined under the microscope, no hydroids, radiolaria, dinoflagellates or Noctilucæ could be observed, but many desmids, diatoms, worms and infusoria. These forms are not luminous, however.

When squeezed through cheesecloth a luminous extract was obtained, the light coming from points of light in the extract as in the case of *Cavernularia* or medusæ. Addition of fresh water or saponin causes a great increase in light just as in extracts of cœlenterates. No luciferin or luciferase could be demonstrated.

The fact that an extract of this sponge made by squeezing through cheesecloth remains luminescent for some time is significant. If the light came from small luminous forms living on the sponge, we should expect them to pass through the meshes of the cheesecloth unharmed and then light would appear in the extract only on stimulation by agitation or in some other way. It is also significant that no inhibitive effect of sunlight was to be observed. Sunlight is said to inhibit the luminescence of many small organisms, especially dinoflagellates, which might live on the sponge. In general characters, the extracts so closely resemble those obtained from cœlenterates that I am inclined to believe the light of this species of sponge is a true luminescence.

LITERATURE.

1. **Panceri, P.**
'72 Ann. d. Sc. Nat., XVI.
2. **Harvey, E. N.**
'17 Amer. Journ. Physiol., XLII., 349.
3. **Dahlgren, U.**
'16 Journ. Franklin Inst., Feb.
4. **Allman, G. I.**
'62 Proc. Roy. Soc. Edinb., IV., 518.
5. **Peters, A. W.**
'05 Journ. Exp. Zoöl., II., 103.